3.7. METAL POLLUTION INDEX-BASED WATER QUALITY ASSESMENT OF THE DANUBE RIVER BASIN IN YUGOSLAVIA

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3.7.1. INTRODUCTION

Although fish do not fulfil all requirements for indicator organism, OECD and ICES agreed upon using trace metal concentrations in stationary fish as possible indicators in areas affected by human activities. (Jorgensen and Pedersen, 1994). Also, bioaccumulation studies of persistent substances in aquatic biota (including fish) are required for NPDES permit in United States (US EPA, 1991a). Fish tissue metal content has been, so far, successfully used in estimations of trace metal input into large European and American rivers. (Pujin *et al.*, 1990, Wachs, 1991, 1992/3, Allen-Gil and Martynov, 1995; Chevreuil *et al.*, 1995; Saiki *et al.*, 1995; Carru *et al.*, 1996, Maletin *et al.*, 1996) and lakes (Salanki, 1982, Balogh, 1985, Strip *et al.*, 1990, Spry and Wiener, 1991, Scharenberg, 1994, Kock *et al.*, 1996).

The Danube River Basin is constantly subjected not only to trace metal and other toxic substances input, but to different legislative, enforcement measures and monitoring programmes along its flow. Therefore, it is becoming inevitable to establish internationally standardised methods for water quality monitoring, which seems not to be a problem when considering physico-chemical analysis of sediments, waters and wastewater. However, biological monitoring and, to a certain extent, ecotoxicological studies are highly dependent on biodiversity and species richness within the region. In spite of the fact that the fish tissue preparation methods, and analytical methods for metal determinations as well, have been standardised so far, (APHA, 1989; U.S. EPA, 1991), the question of choosing the most appropriate fish group, species and tissue for these kind of monitoring studies is still to be answered. Therefore, the objective of this paper is to contribute to ecotoxicological studies of the Danube River by offering applicable method for standardised monitoring of metal pollution in the Basin. Recently introduced Metal Pollution Index (Teodorovic *et al.*, 1998b, Teodorovic *et al.*, 1999, Teodorovic, 1999) has been calculated for selected ecosystems within the lower Danube River Basin with an aim to produce some useably data for comparative, as well as monitoring purposes, at least within the region.

3.7.2. MATERIAL AND METHODS

3.7.2.1. Sampling and chemical analysis

The fish for this study was caught in spring and summer 1997 from the River Danube (1 sampling site), from The Danube wetlands (2), Canal System Danube - Tisza - Danube (5) , the River Jegricka (1), Backwater Tisza (1) , Zasavica (Backwater Sava) (1) and 3 reservoirs within the Basin (Fig 1).

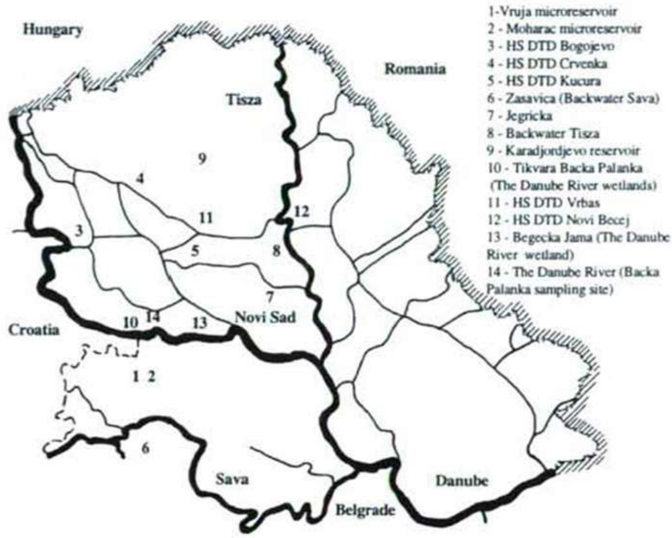

Fig. 1 Sampling stations

All samples consisted of 10 specimens of *Carassius auratus gibelio*, except the samples from Zasavica (backwater Sava) where *Carassius carassius* was taken. Tissue (fish liver) digestion and sample preparation was done according to standard procedure (U.S. EPA, 1991) and Perkin Elmer AAS (flame and graphite furnace with background correction) was used for Cd, Zn, Cu, Pb and Al determinations. (APHA, 1989). All results are presented on a wet weight basis, as mg/kg, but recalculation by the factor 2.6 from wet to dry weight basis for liver enables comparison.

3.7.2.2. Metal Pollution Index (MPI)

MPI has been calculated to enable presentation of all results from 5 metal concentrations (Cd, Cu, Zn, Pb and Al) as one value if possible, yet overcoming the difficulties with both application and understanding of demanding statistical analysis. According to Jorgensen and Pedersen (1994), this implies that the five metal concentrations must be normalised to make it possible to sum up and average the different metal concentrations into one value and, what is more important, to diminish the more than thousand-fold difference between the least and the most abundant elements. Without such transformation, the least abundant elements would be without influence on the results. (Julshamn and Grahl-Nielsen, 1996). We have chosen the average values of fish liver metal burden (Cu, Zn, Pb, Al and Cd) from the reference sites. (Vruja and Moharac microreservoir) Such normalizer is used to account for the biological variation in a non-polluted area. Moreover, as seasonal variations in metal content have been observed (Balogh *et al.*, 1985; Kock *et al.*, 1996), sampling was undertaken within the same season (spring/summer), at precisely defined sampling stations (Fig 1).

Since no significant difference has been found between liver metal content in goldfish from two microreservoirs (one way ANOVA, $p \leq 0.05$) (Teodorovic, 1999) the sample has been pooled so the reference values represent the means$\pm$ SD of 20 specimens. (Table 1).

Table 1 Concentrations (mg/kg wet wt) of Cd, Pb, Zn, Cu and Al in goldfish liver from Vruja and Moharac microreservoirs and proposed referent values (normalizers) * below detection limit (0.015 ppm)

	Cd	Pb	Zn	Cu	Al
pooled sample Vruja & Moharac	*	0.19± 0.42	19.58± 0.7	1.91±0.42	13.62 ±4.01
proposed normalizers	0.015	0.2	20	2	15

MPI has been calculated as:

$$MPI = \log \sum_{i=1}^{n=5} \frac{[\bar{x}]}{ref_i} \qquad (1)$$

where ref_i represents a normalizer, or a reference value for each of five chosen metals (Cd, Cu, Pb, Zn and Al) in liver, while x represents mean value ($n \geq 10$, SD up to 30%) of metal concentration in the same tissues from the chosen sampling site). Furthermore, logarithmic transformation enables normalisation of MPI values. If calculated as proposed, MPI distinguishes: non polluted if MPI< 1, polluted: 1<MPI<1.5, and very polluted ecosystem: if this combined index is above 1.5. In other words, ecosystems are being classified into three categories: "non-polluted" - if the sum of the normalised metal concentrations is up to twice the reference value, "polluted" - if from 2 to 6 time higher and "very polluted" - if the sum of normalised metal concentration is more than 6 time higher than the reference value.

3.7.3. Results and Discussion

Trace metal accumulation patterns in aquatic biota (Strip *et al.*, 1990, Pujin *et al.*, 1990, Wachs, 1992/3, Allen, 1995, Kraal *et al.*, 1995), along with bioconcentration (Salanki *et al.*, 1982, Wachs 1990, Kock *et al.*, 1995 Djukic *et al.*, 1998a,b) and biomagnification processes (Jorgensen and Pedersen, 1994, Saiki *et al.*, 1995, Carru *et al.*, 1996, Teodorovic *et al.*, 1998a,b) have been excessively studied. The general conclusions regarding the named processes are that muscle is the tissue of the lowest, while liver, kidney and gills represent the target tissues for trace metal accumulation in fish. To minimise expenses and, on the other hand, to standardise procedure, fish liver was chosen as the monitoring tissue in this study. Further on, researchers mainly agree upon the fact that Hg is the only metal which is subjected to biomagnification via aquatic food chain. Nevertheless, bentivore fish proved to accumulate Cd and Pb to higher extent than the piscivore, which is to be expected considering biology and feeding habits of the group. To avoid possible species-specific differences, *Carassius auratus gibelio*, which, according to recently published data (Jankovic, 1994; Maletin *et al.*, 1997) makes up to 50% out of total catch in Yugoslav part of the Danube Basin, has been taken as sentinel species.

Besides, to diminish possible age/size influence on trace metal content, only specimens belonging to same age group (1+) have been used in this study.

Table 2 shows Zn, Cu, Cd, Pb and Al concentrations (mg/kg wet wt) in goldfish (*Carassius auratus gibelio*) liver from 12 representative study sites within the Yugoslav part of the Danube River Basin. The content of all analysed metals was the lowest in fish liver from Vruja and Moharac microreservoirs. (table 1). Such results were expected as these microreservoirs do not receive any direct wastewater (sewage nor industrial) input and are quite remote from urban areas and major roads. Moreover, when compared with the results of previous research of trace metal load in bentivore fish (Pujin *et al.*, 1992, Maletin *et al.*, 1992, Maletin *et al.*, 1996, Djukic *et al.*, 1998a,b) these concentrations are significantly the lowest ever recorded in Yugoslav part of the Danube Basin. (Teodorovic, 1999). Therefore, as explained in Material and Methods section, these values have been chosen as normalizers (reference values).

Table 2 Metal concentrations (mg/kg wet wt) in goldfish (*Carassius auratus gibelio*) liver (mean n=10 ±SD) from representative study sites within the Yugoslav part of the Danube River Basin

ecosystem/ sampling site	Zn	Cu	Cd	Pb	Al
HS DTD Bogojevo	20.46 ±2.3	2.24± 0.42	*	0.2 ±0.02	15.34 ±1.67
HS DTD Crvenka	31.58± 0.47	2.21± 0.1	0.01	0.39± 0.1	16.09 ±0.16
HS DTD Kucura	30.41± 3.67	3.34± 1.09	0.02	0.44 ±0.09	15.11 2.37
Zasavica	21.22±1.96	5.59±0.32	*	0.9±0.28	17.98±7.85
Jegricka - Zabalj	46.87± 26.18	2.1± 0.34	0.01	0.79± 0.38	84.9 ±12.4
Backwater Tisza - Curug	34.9±7.69	10.07±2.85	0.11±0.05	1.8±0.41	21.08±5.64
Karadjordjevo reservoir	75.03±20.1	2.7±0.2	0.13±0.07	1.96±0.21	35.23±4.12
Tikvara Backa Palanka (The Danube wetland)	57.46± 3.69	9.22± 2.55	0.19± 0.08	0.95± 0.21	17.87± 7.13
HS DTD Vrbas	30.79 ±0.19	16.75 ±1.41	0.13± 0.01	0.59 ±0.12	107 ±29
HS DTD Becej	34± 9	13.7± 2.5	0.2± 0.05	3± 0.6	64 ± 3
Begecka Jama (The Danube wetlands)	225± 90	9.07± 5.7	0.23± 0.11	1.96± 0.03	45.84± 7.27
The Danube River (Backa Palanka)	123± 20	8.71 ±3.2	0.26± 0.07	5.18 ±0.5	15.81± 8.4

The Metal Pollution Index for selected sites in Yugoslav part of the Danube River Basin has been calculated according to equation (1). The results are presented in Fig. 2, where MPI based classification into 3 categories has also been shown. The lowest values of MPI are recorded at three study sites Bogojevo, Crvenka and Kucura along the Danube - Tisza- Danube Canal System, 0.72, 0.83 and 0.89, respectively. According to MPI-based classification, these sites could be regarded as non-polluted, considering trace metals. The highest values (1.61, 1.64 and 1.73) are recorded at Becej dam, in the Danube wetland Begecka Jama (upstream Novi Sad) and in the Danube River (sampling site Backa Palanka), respectively. These are the sites that, according to MPI value, must be considered as very polluted. Between these two extremes, there are 6 ecosystems: Zasavica (Backwater Sava), the Jegricka River (sampling site Zabalj), Backwater Tisza (sampling site Curug), Reservoir Karadjordjevo, Tikvara - Backa Palanka (The Danube wetland upstream Novi Sad) and Vrbas (Canal System Danube-Tisza-Danube) with MPI values ranging from 1.02 to 1.46, and consequently classified as polluted.

The sampling sites with the highest MPI : Backa Palanka (The Danube -1.73 and the wetlands 1.42) and Begecka Jama wetland (1.64) are located on The Danube River section between Vukovar (Croatia) and Novi Sad. This area is urbanised and industrialised, so the Danube River receives huge amounts of untreated and partly treated municipal and industrial effluents, particularly from metal processing, leather and textile industry. The

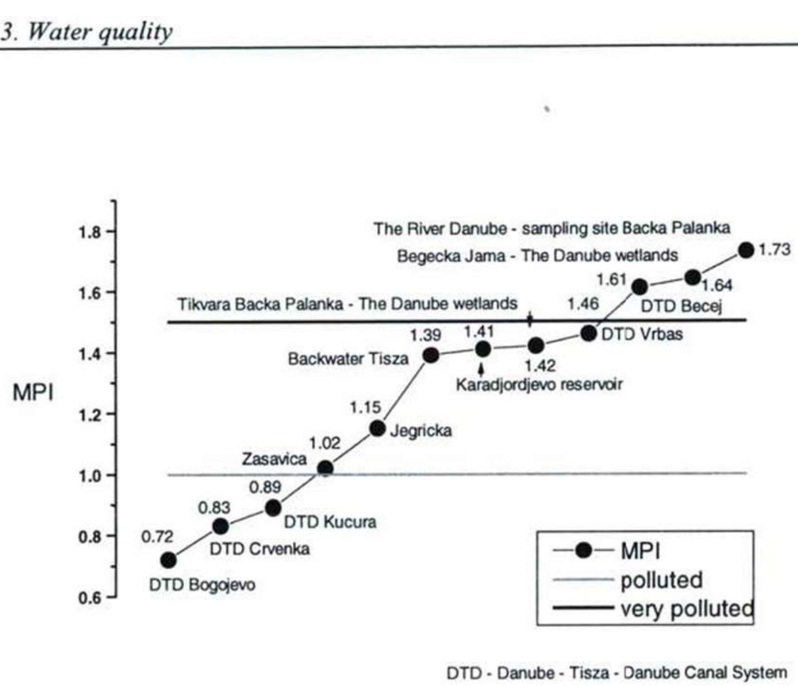

Fig 2. MPI-based evaluation of the selected ecosystems within the Yugoslav part of the Danube River Basin

results of Yugoslav national monitoring programme (chemical analysis of water and sediments) from this section of the Danube River in period 1995-1997 (Anon, 1995, 1996, 1997) reveal highly changeable concentrations of Zn (0-30 μg/l), Pb (0-36μg/l) and Cu (0-12μg/l) in water, while most of the time Cd was below the detection limit. On the other hand, sediment concentrations of Zn and Cu ranged from 30 - 200 mg/kg, while Cd and Pb were constantly below the detection limit. Yet, Cd and Pb are the main components of such high MPI values since their average concentrations in goldfish liver from these sites are 12-15 and 5-26 time higher than reference values, respectively. The similar situation is found in Canal section Vrbas - Bezdan on sampling site Vrbas (MPI 1.46) located downstream effluent discharges of industrial zone Kula - Vrbas (food, metal processing, leather and textile industry). Trace metal concentrations in Canal water show even higher variability than in the Danube. (Zn 10-180000 μg/l; Cd 0-5, Pb 0-26, Cu 0-3 μg/l), (Anon, 1995, 1996, 1997), while Cd and Cu contents represent the main components of the MPI, being 8 times higher than the reference values. These results prove the existing opinion that accumulated trace metal content in aquatic biota has smaller variability and thus could serve as more reliable indicator of the metal pollution than chemical analysis of water and sediments. (Salanki *et al.*, 1982, Sharenberg *et al.*, 1994).

The "non-polluted" sites along the Canal system (Bogojevo, Crvenka and Kucura), as well as "polluted" Backwater Tisza (Zabalj) and Zasavica are located in predominantly rural areas, so that trace metal occurrence in aquatic environment is due only to patchy inputs, mainly runoff from agricultural land.

However, the results of this study must be taken with the utmost care. The reason for such statement is the fact that due to economic and political situation in Yugoslavia, industrial facilities in the whole region have not been working with full capacity for almost 10 years and the river transport, due to the latest events, is insignificant compared to the period before 1990. Therefore, toxic substances (including trace metals) inputs to the Danube River Basin from the Yugoslav and Croation territories is impared. Yet, MPI-based evaluation indicates metal pollution as an already serious environmental issue. Thus, it could be expected that in future, after the normalisation of the situation in the region, the problem of toxic substances input emerges as one of the hot spots in the lower part of the River Danube. Our opinion is, therefore, that MPI could serve in future for trend analysis and comparative purposes as an easy, relatively prompt and inexpensive monitoring method.

3.7.4. SUMMARY

MPI - Metal Pollution Index is being introduced with an aim of improving the freshwater pollution control, monitoring and classification based on fish metal body burden. This simple mathematical model enables presentation of Cd, Pb, Cu, Zn and Al fish liver concentration as a single unit-less value and, therefore, evaluation, classification and time trend analysis within the region. Metal burden in liver of *Carassius auratus gibelio* has been used for calculations, while normal distribution and biological variation estimation have been achieved by using normalizers (liver concentrations of selected elements in fish from reference sites). MPI - based water quality assessment and classification of the selected sites within the Yugoslav part of the Danube River Basin have been undertaken. The results of this study indicate that metal pollution of the Danube River could be regarded as a rather serious environmental issue.

3.7.5. ACKNOWLEDGEMENTS

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